

Influence of protein supplementation frequency on cows consuming low-quality forage: Performance, grazing behavior, and variation in supplement intake¹

C. S. Schauer^{*2}, D. W. Bohnert^{*3}, D. C. Ganskopp[†], C. J. Richards[‡], and S. J. Falck[†]

^{*}Eastern Oregon Agricultural Research Center, Oregon State University, and

[†]ARS, USDA, Eastern Oregon Agricultural Research Center, Burns 97720; and

[‡]Animal Science Department, University of Tennessee, Knoxville 37996

ABSTRACT: The objectives of this research were to determine the influence of protein supplementation frequency on cow performance, grazing time, distance traveled, maximum distance from water, cow distribution, DMI, DM digestibility, harvest efficiency, percentage of supplementation events frequented, and CV for supplement intake for cows grazing low-quality forage. One hundred twenty pregnant (60 ± 45 d) Angus \times Hereford cows (467 ± 4 kg BW) were used in a 3×3 Latin square design for one 84-d period in each of three consecutive years. Cows were stratified by age, BCS, and BW and assigned randomly to one of three 810-ha pastures. Treatments included an unsupplemented control (CON) and supplementation every day (D; 0.91 kg, DM basis) or once every 6 d (6D; 5.46 kg, DM basis) with cottonseed meal (CSM; 43% CP, DM basis). Four cows from each treatment (each year) were fitted with global positioning system collars to estimate grazing time, distance traveled, maximum distance from water, cow distribution, and percentage of supplementation events frequented. Collared cows were dosed with intraruminal n-alkane controlled-release devices on d 28 for estimation of DMI, DM digestibility, and harvest

efficiency. Additionally, Cr_2O_3 was incorporated into CSM on d 36 at 3% of DM for use as a digesta flow marker to estimate the CV for supplement intake. Cow BW and BCS change were greater ($P \leq 0.03$) for supplemented treatments compared with CON. No BW or BCS differences ($P \geq 0.14$) were noted between D and 6D. Grazing time was greater ($P = 0.04$) for CON compared with supplemented treatments, with no difference ($P = 0.26$) due to supplementation frequency. Distance traveled, maximum distance from water, cow distribution, DMI, DM digestibility, and harvest efficiency were not affected ($P \geq 0.16$) by protein supplementation or supplementation frequency. The percentage of supplementation events frequented and the CV for supplement intake were not affected ($P \geq 0.58$) by supplementation frequency. Results suggest that providing protein daily or once every 6 d to cows grazing low-quality forage increases BW and BCS gain, while decreasing grazing time. Additionally, protein supplementation and supplementation frequency may have little to no effect on cow distribution, DMI, and harvest efficiency in the northern Great Basin.

Key Words: Cattle, Distribution, Grazing Behavior, Northern Great Basin, Protein Supplementation, Sagebrush Steppe

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Introduction

Cattle grazing in the northern Great Basin of the western United States typically consume low-quality forage ($<6\%$ CP, DM basis) from late summer through winter; therefore, protein supplementation is necessary to maintain or increase cow BW and BCS (Clanton and Zimmerman, 1970; Rusche et al., 1993). Protein supplementation can be expensive; however, decreasing the frequency of supplementation can decrease labor costs. Melton and Riggs (1964) suggested that providing cottonseed cake (approximately 4 kg) twice per week resulted in savings of approximately 60% in labor and

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²Current address: Hettinger Res. Ext. Center, North Dakota State Univ., Hettinger 58639.

³Correspondence: 67826-A Hwy 205 (phone: 541-573-8910; fax: 541-573-3042; e-mail: dave.bohnert@oregonstate.edu).

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travel when compared with daily supplementation. Research has shown that protein supplements can be offered infrequently to ruminants with acceptable performance maintained compared with daily protein supplementation (McIlvain and Shoop, 1962; Huston et al., 1999b; Bohnert et al., 2002b).

A review of relevant research indicates that grazing time decreases by 1.5 h/d for cottonseed meal-supplemented (0.22 to 0.25% of BW/d) vs. unsupplemented cows (Krysl and Hess, 1993). However, little research has addressed the effects of supplementation frequency on livestock distribution and grazing behavior. Therefore, our objectives were to determine whether infrequent protein supplementation to cows grazing low-quality forage affects cow performance, grazing time, distance traveled, maximum distance from water, cow distribution, DMI, DM digestibility, harvest efficiency, percentage of supplementation events frequented, and the CV for supplement intake.

Materials and Methods

Experimental Site

Research was conducted at the Northern Great Basin Experimental Range (lat 119°43'W, long 43°29'N; elevation 1,425 m), 72 km west-southwest of Burns, OR. Climate at the study area is characterized by marked seasonal variations in both temperature and precipitation. Mean annual precipitation is 28.3 cm, and average growing season (May through September) temperature is 15.5°C (average minimum = 6.6°C; average maximum = 24.4°C). Vegetation is a dispersed western juniper (*Juniperus occidentalis* Hook.) overstory and a shrub layer dominated by either low sagebrush (*Artemisia arbuscula* Nutt.), Wyoming big sagebrush (*A. tridentata* subsp. *wyomingensis* Beetle), or mountain big sagebrush (*A. tridentata* subsp. *vaseyana* [Rydb.] Beetle). Dominant herbaceous plants included bluebunch wheatgrass (*Agropyron spicatum* [Pursh] Scribn. and Smith), Idaho fescue (*Festuca idahoensis* Elmer), and Sandberg's bluegrass (*Poa sandbergii* Vasey; Ganskopp, 2001). The diversity of graminoid and shrub species indicates a diversity of soil types (54 map units within the study area). The majority of the soils are Ninemile cobbly loam complexes, ranging from shallow clay loams to deeper sandy loams (<6 cm), combined with rock outcroppings.

Experimental Design

One hundred twenty pregnant (60 ± 45 d) Angus \times Hereford cows (467 ± 4 kg initial BW) were used in a 3×3 Latin square with one 84-d period in each of three consecutive years (2000, 2001, and 2002) to evaluate the influence of supplementation frequency on cow BW and BCS change. Additionally, a subsample of 12 cows from the larger herd was used within the same design and periods to evaluate the influence of supplementa-

tion frequency on cow grazing time, distance traveled, maximum distance from water, distribution within pasture, DMI, DM digestibility, harvest efficiency, percentage of supplementation events frequented, and the CV for supplement intake. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Oregon State University. Cows were stratified by age (4.6 ± 1.3 yr), BCS (4.4 ± 0.5 ; 1 = emaciated, 9 = obese; Herd and Sprott, 1986), and BW (435 ± 47 kg) before being assigned randomly to one of three pastures (810 ha/pasture). The same cows were used in successive years; however, if an animal did not complete the experiment (death or not pregnant at weaning), another animal of similar age and genetic background replaced it. Cows were not rotated through the pastures; each cow group remained in the same pasture for all 3 yr. During the approximately 9 mo between experimental periods, all cows were grouped into one herd and managed according to Northern Great Basin Experimental Range and Eastern Oregon Agricultural Research Center management practices. No grazing by domestic livestock occurred in the pastures between experimental periods. Treatments included an unsupplemented control (**CON**) and supplementation every day (**D**; 0.91 kg, DM basis) or once every 6 d (**6D**; 5.46 kg, DM basis) with cottonseed meal (**CSM**; 43% CP, DM basis). Cottonseed meal was provided 10 min after an audio cue (Signal Horn, Tempo Products Co., Solon, OH) at approximately 0800 for each supplementation event. The cottonseed meal was evenly distributed within troughs, with approximately 75 cm of trough space allocated per cow. A loose trace mineralized salt mix was available free choice (27.8% Na, 23.1% Cl, 7.3% Ca, 7.2% P, 1.7% Mg, 1.5% K, 0.5% S, 3,202 ppm Zn, 3,034 ppm Fe, 2,307 ppm Mn, 1,340 ppm Cu, 85 ppm Se, 78 ppm I, 32 ppm Co, 397 kIU/kg vitamin A, and 79 IU/kg vitamin E).

Pasture average minimum and maximum elevations were 1,402 and 1,621 m, respectively. Pastures were square, with water, mineral/salt, and supplement placement centrally located and maintained in the same location throughout the experiment within each pasture, both at the easting/northing center and elevation median (one location in each pasture). Supplement troughs were 50 m from water.

Experimental periods were 84 d, beginning August 9 (2 wk after weaning) and concluding November 1 of each year. Cow BW and BCS were measured on d 0 and 84 following an overnight shrink (16 h). Cow BCS was judged independently by three observers (Herd and Sprott, 1986). The same technicians measured BCS throughout the experiment. Supplement samples were collected weekly, dried at 55°C for 48 h, ground in a Wiley mill (1-mm screen), and composited by period for analysis of DM, OM (AOAC, 1990), and N (CN-2000, Leco Corp., St. Joseph, MI).

Forage Nutritive Value

Five 4-yr-old ruminally cannulated Angus \times Hereford steers (365 ± 23 kg) were used to quantify forage nutri-

tive value in each pasture using three 2-ha fenced enclosures similar in botanical composition to the larger 810-ha pastures (one in each pasture) on d 1 through 3, 43 through 45, and 80 through 82, except for yr 1 when data were not collected for d 1 through 3. The 2-ha enclosures were used because of the logistical and topographical constraints associated with allowing the cannulated steers to graze with the experimental cows within the 810-ha pastures. The majority of grasses were in senescence during the period when the study occurred; therefore, forage quality and quantity across the landscape were assumed to not be highly variable. Additionally, the regions in which the 2-ha enclosures were located were historically grazed as part of the 810-ha pastures. Available standing forage was determined by clipping before each collection period to ensure adequate forage availability (data not shown). Actual forage production in enclosures was 85% of forage production in the 810-ha pastures (data not shown). The use of enclosures as a subsample of the larger pastures may have biased data; however, due to the size and nature of the study, we feel this technique allowed for adequate data collection that was representative of each respective pasture. Masticate samples were collected from one enclosure each day during the morning grazing bout of the respective cows in the 810-ha pasture (observational data). Steers were caught at 0730, transported to an enclosure, and ruminally evacuated (not previously withheld from grazing) as described by Lesperance et al. (1960), except that the ruminal wall was washed with a wet sponge, and steers were allowed to graze for approximately 1 h. The newly grazed masticate was removed, and the initial ruminal contents were replaced. Beginning 2 wk before study initiation, and when not being used to collect masticate samples, steers grazed a pasture containing forage species similar to those in the experimental pastures. Masticate was immediately frozen (-20°C), lyophilized, and ground in a Wiley mill (1-mm screen) for determination of DM, OM (AOAC, 1990), N (CN-2000), NDF (Robertson and Van Soest, 1981), and ADF (Goering and Van Soest, 1970) using procedures modified for an Ankom 200 fiber analyzer (Ankom Co., Fairport, NY).

Standing Crop

Available standing crop in each pasture was measured at the beginning and conclusion of each experimental period (d 0 and 84, respectively) by clipping graminoid and forb species in 10 randomly placed 1-m² quadrats from representative plant communities in each pasture. All vegetation types described previously were evaluated in proportion to their presence within each pasture. Because of senescence, however, only trace amounts of forbs were present; therefore, these data are not reported separately from graminoid data. Range sites were selected based on known soils and associated vegetation within each pasture (Lentz and Simonson, 1986), as well as on known historic grazing

patterns (data not shown). In addition, standing crop in each 2-ha enclosure was estimated by clipping (as described above) immediately before and after each fecal collection period (described in DM and supplement intake section). Clipped herbage was dried at 55°C for 48 h and weighed for determination of standing crop.

Cow Distribution and Behavior

Cows were stratified by age within treatment, and four cows from each treatment (each year) were assigned randomly within stratification to be fitted with a global positioning system (GPS) collar (GPS 2000 collars, Lotek, Newmarket, Ontario, Canada) to obtain data related to animal distribution and behavior. Collars were placed on the same cows each year. If an animal was removed from the study (death or not pregnant at weaning), another animal of similar age and genetic background replaced it the following year. Collars were equipped with head forward/backward and left/right movement sensors, a temperature sensor, and a GPS unit. Collars were programmed to take position readings at 10-min intervals for three 6-d periods (in each of three consecutive years) to estimate grazing time (h/d), distance traveled (m/d), maximum distance from water (m/d), cow distribution (% of ha occupied $\cdot \text{pasture}^{-1} \cdot \text{yr}^{-1}$), and percentage of supplementation events frequented. All treatments were monitored during the same 6-d periods (d 18 through 23, 48 through 53, and 72 through 77) each year. The 6-d periods were scheduled to encompass a complete supplementation cycle for the 6D treatment. Collar data were retrieved after each 6-d period, downloaded to a computer, and converted from latitude/longitude to Universal Transverse Mercator as described by Ganskopp (2001). Grazing time was determined through generation of a prediction model for each cow, each year. Briefly, each collared cow was visually observed for 10 ± 2 h per year. During these visual observation periods, the collared cows were at all times observed in the presence of at least part of the herd, never by themselves. Activities monitored included grazing, resting (lying down), walking, standing, drinking, and consuming mineral or supplement. Prediction models for estimating grazing time were developed via stepwise regression analysis ($\text{SLENTY} = 0.25$; $\text{SLSTAY} = 0.15$) for each cow/year (SAS Inst., Inc., Cary, NY). The dependent variable was grazing time (min/10-min interval), and the independent variables from GPS collar data included head forward/backward and left/right movement sensor counts, sum of forward/backward and left/right movement counts, and the distance traveled (m) by the cow within each 10-min interval. Individual models for each cow $\cdot \text{treatment}^{-1} \cdot \text{year}^{-1}$ were used to evaluate treatment differences for grazing time (Schauer, 2003). Distance traveled (used for determining grazing time and distance traveled/d) was most likely underestimated because straight-line pathways were assumed between successive coordinates. Cow distribution within pasture

was determined with Geographical Information Systems software (Idrisi32 for Windows, Clark University, Worcester, MA), using 1-ha grid cells within each pasture (% ha occupied \cdot pasture⁻¹ \cdot yr⁻¹). The percentage of supplementation events frequented was determined as the percentage of collared cows within 50 m of supplement, within 20 min of a supplementation event. This technique was used to determine whether group feeding behavior was modified by decreasing supplementation frequency.

Dry Matter and Supplement Intake

Each GPS-collared cow was dosed with an intraruminal n-alkane controlled-release device (IACRD; Captec Ltd., Auckland, New Zealand) containing dotriacontane (C32) and hexatriacontane (C36) on d 28. Dotriacontane and tritriacontane (C33) were used as digesta flow markers to estimate DMI and digestibility as described by Dove and Mayes (1996). Additionally, chromic oxide was mixed with the CSM (meal form) in a cement mixer on d 36 (day of supplementation for D and 6D treatments) at 3% of supplement DM for use as a digesta flow marker to estimate supplement intake. Vegetable oil was added at 2.5% of supplement DM to minimize dust and to facilitate uniform distribution of Cr throughout the supplement. A subsample of the Cr-CSM mixture was collected for later analysis of Cr. All 40 cows per treatment were herded to supplement bunks and allowed the opportunity to consume chromic oxide dosed supplement. This technique was used (vs. the audio cue) to ensure that all 40 cows were present at the supplementation event when chromic oxide was administered. Although this was the only day that the cows were driven to supplement, we believe it was an accurate technique for measuring the variability in supplement intake when all 40 cows were present, allowing this portion of the data to be extrapolated and used beyond the northern Great Basin, regardless of pasture size. Because of logistical constraints, it was not possible to sample all 40 cows per treatment for variability in supplement intake. After all supplement was consumed on d 36, the four IACRD-dosed cows per treatment were placed in the 2-ha enclosures used to determine forage nutritive value to facilitate fecal collection. Only four cows per treatment were evaluated for DMI and digestibility and variability in supplement intake. The logistics of conducting serial fecal collections did not allow for collection from a larger number of animals or for multiple collection periods. Fecal grab samples (approximately 300 g) were obtained from IACRD-dosed cows on d 36 through 40 at 0800 h to determine n-alkane concentration for estimation of DMI and digestibility. In addition, fecal grab samples from D and 6D IACRD-dosed cows were collected at 0, 12, 24, 28, 32, 40, 48, 54, 60, 72, 84, and 96 h following supplementation to derive a dose response curve for fecal Cr concentration (cows were released to pasture on d 40 following the 96 h fecal collection). Fecal samples were dried

at 55°C for 96 h and ground in a Wiley mill to pass a 1-mm screen. The fecal samples obtained from IACRD-dosed cows at 0800 on d 36 through 40 were composited by cow within year for analysis of n-alkanes, whereas the serially collected D and 6D fecal samples were analyzed individually for Cr.

The IACRD release rates of C32 and C36 alkanes were validated using four steers consuming low-quality forage (<6% CP, DM basis). All IACRD used in the study were from the same production lot; therefore, only one validation study was conducted. Total intake, ort, and fecal collections were conducted on five consecutive days (d 10 to 14 following dosing). Meadow hay was used for the validation study because an accurate determination (not an estimate) of intake was necessary to determine IACRD alkane payout. Therefore, steers were fed meadow hay ad libitum at 120% of the previous 5-d average intake. Fecal samples were dried at 55°C for 96 h and ground in a Wiley mill to pass a 1-mm screen. Masticate samples from cannulated steers and hay, supplement, ort, and fecal samples were analyzed for n-alkanes. Feed (masticate, hay, and orts) and fecal samples were weighed into 50-mL glass screw-cap tubes with Teflon-lined caps (1.5 and 1.0 g, respectively). One milliliter of internal standard (0.25 mg/mL of tetratriacontane; C34) was placed in each tube, followed by 15 mL of 1 M ethanolic KOH per tube. Samples were mixed and incubated in a shaking water bath at 90°C for 8 h, and vortexed at least four times during the incubation. After tubes cooled to 60°C or less, 14 mL of n-heptane and 4 mL of distilled water were added, and tubes were capped and placed in a 60°C shaking water bath for 2 h. Tubes were then shaken vigorously on a shaker at room temperature for 18 h and centrifuged at 500 \times g for 15 min at 4°C. The top layer (n-heptane layer) was then transferred with a glass Pasteur pipette into a 16 mm \times 125 mm Pyrex tube and evaporated until dry in a CentriVap centrifugal concentrator (Labconco, Kansas City, MO) at 75°C. Residual material in the centrifuge tube was subjected to a second n-heptane extraction as above, with the n-heptane layer added to the Pyrex tube containing the evaporated material from the previous extraction and evaporated until dry. The sample was reconstituted with 3.5 mL of n-heptane and vortexed until dissolved. A solid-phase separation was conducted by pouring the reconstituted sample through a chromatography column packed with 5 mL of Porasil silica (125 Å, 55 to 105 μ m; Waters, Corp., Milford, MS). The column was rinsed three times with 3.5 mL of n-heptane, and the combined eluants were collected. Samples were evaporated as previously noted. Feed and fecal samples were reconstituted with n-heptane (0.25 and 0.5 mL, respectively), transferred to gas chromatograph vials using glass Pasteur pipettes, capped, and sealed. Samples were analyzed by gas chromatography on a HP 5890 (Agilent Technologies, Palo Alto, CA) chromatograph, with a flame-ionization detector, a Supelco (Bellefonte, PA) SPB-1 wide-bore capillary column (30 m long \times 0.75 mm i.d. \times 1 μ m film thickness), and

helium as the carrier gas (20 mL/min). Column temperature was set at 280°C, with injector and detector temperatures of 325 and 350°C, respectively.

Alkane concentrations for meadow hay in the validation study were 0.018, 0.03, 0.017, and 0.014 mg/g for C32, 33, 35, and 36, respectively. Alkane concentrations for masticate samples from the 2-ha enclosures were 0.032, 0.06, 0.03, and 0.001 mg/g for C32, 33, 35, and 36, respectively. Validated release rates for C32 and C36 alkanes were 240 ± 3 and 242 ± 11 mg/d, respectively, which were $60 \pm 1\%$ and $61 \pm 3\%$ of the predicted release rate, respectively. These release rates are consistent with other trials conducted with low-quality forage (E. S. Vanzant, University of Kentucky, personal communication). Fecal recovery of naturally occurring C33 and C35 were 98 ± 6 and $79 \pm 2\%$, respectively; therefore, DMI ($\text{g} \cdot \text{kg} \text{ BW}^{-1} \cdot \text{d}^{-1}$) was determined through the ratio of dosed C32 and naturally occurring C33 concentrations ($[\{\text{fecal}_{\text{C33}}/\text{fecal}_{\text{C32}}\} \times \text{dosed}_{\text{C32}}]/[\text{herbage}_{\text{C33}} - \{\{\text{fecal}_{\text{C33}}/\text{fecal}_{\text{C32}}\} \times \text{herbage}_{\text{C32}}\}]$), whereas DM digestibility (%) was determined using the ratio of herbage and fecal C33 concentrations ($1 - [\text{herbage}_{\text{C33}}/\text{fecal}_{\text{C33}}]$; Dove and Mayes, 1996). Fecal output was calculated from intake and digestibility data determined from n-alkane analysis.

Supplement and fecal samples were prepared as described by Williams et al. (1962) for analysis of Cr using atomic absorption spectroscopy (air/acetylene flame; model 351 AA/AE spectrophotometer, Instrumentation Laboratory, Inc., Wilmington, MA). Fecal Cr concentration and fecal output derived from alkane data were used to estimate the dose of Cr by an algebraic approach: dose of Cr (μg) = (fecal output/h) \times area under the Cr excretion curve (Galyean, 1993). The area under the Cr excretion curve was calculated as follows: area under the curve ($[\mu\text{g of Cr/g of fecal DM}] \times \text{h}$) = $(\sum c_i + c_p) \times (t_i - t_p)/2$, where c_i = the current concentration ($\mu\text{g of Cr/g of fecal DM}$), c_p = the concentration at the previous collection time ($\mu\text{g of Cr/g of fecal DM}$), t_i = the current collection time (h), and t_p = the previous collection time (h). Supplement intake was calculated as Cr dose/Cr concentration in supplement. The CV for supplement intake was determined from estimated supplement intake. Harvest efficiency was calculated as grams DMI $\cdot \text{kg} \text{ BW}^{-1} \cdot \text{min}$ spent grazing $^{-1}$. Dry matter intake from the IACRD-dosed cows was used to calculate harvest efficiency for all GPS collection periods.

Statistics

Latin square designs assume an absence of interactions among rows, columns, and treatments (Ostle, 1963). Therefore, forage quality data were analyzed to determine whether such interactions existed (Krysl et al., 1989). To test year effects, a model was used that included year, treatment, and year \times treatment. Only masticate OM showed a significant year \times treatment interaction ($P = 0.01$) or treatment effect ($P < 0.01$).

Masticate NDF, ADF, and CP did not exhibit a significant year \times treatment interaction or treatment effect ($P \geq 0.37$). Although NDF, ADF, and CP exhibited a year effect ($P < 0.01$), it was concluded that the nature of the responses (no interactions) did not preclude analyzing the data as a 3×3 Latin square. In addition, Krysl et al. (1989) noted that Latin square designs are used infrequently in range studies because of the potential row/column effects; however, treatment comparisons are valid but tend to be conservative in nature because of potential interactions.

Forage nutritive value, available standing crop, cow BW and BCS change, distribution within pasture, DMI, DM digestibility, harvest efficiency, percentage of supplementation events frequented, and CV for supplement intake were analyzed as a 3×3 Latin square using the GLM procedure of SAS. The model included treatment, year, and pasture (Krysl et al., 1989). Orthogonal contrasts, CON vs. supplemented treatments, and D vs. 6D, were used to partition specific treatment effects. Grazing time, distance traveled, and maximum distance from water, determined for each day of the three 6-d GPS data collection periods, were averaged by day within year and analyzed using the Repeated statement with the Mixed procedure of SAS. The model included pasture, year, treatment, day, and treatment \times day. Pasture \times year \times treatment was used to specify variation between experimental units (using the Random statement). Pasture \times year \times treatment was used as the Subject and an autoregressive covariance structure was assumed. The same orthogonal contrasts noted above were used to partition treatment sums of squares.

Results and Discussion

Standing Crop and Nutritive Value

Initial standing crop was unaffected ($P \geq 0.11$) by treatment, pasture, or year (299 ± 27 kg/ha); however, ending standing crop tended to decrease ($P = 0.06$) from yr 1 through 3 ($266, 170$, and 164 ± 15 kg/ha, respectively), with no effect ($P \geq 0.11$) of treatment or pasture. Initial standing crop was not affected ($P \geq 0.30$) by treatment, pasture, or year (251 ± 72 kg/ha) in the fecal collection enclosures, but similar to the results with the pastures, final ending standing crop in nutrition enclosures successively decreased ($P = 0.04$) from yr 1 through 3 ($288, 171$, and 122 kg/ha, respectively), with no difference ($P \geq 0.10$) related to treatment or pasture. Precipitation for the crop year was 86, 72, and 42% of the 65-yr average (WRCC, 2003) for yr 1, 2, and 3, respectively, which may explain the decrease in herbage across years as the trial progressed. Forage nutritive value did not differ among treatments ($P \geq 0.16$); therefore, results are reported by year and pasture (Table 1). Masticate CP concentrations are higher than those reported by Turner and DelCurto (1991; $<5\%$ CP) for similar forage at a comparable time period at the

Table 1. Effect of year and pasture on forage nutritive value of diets selected by steers grazing native range in the northern Great Basin

Masticate	Year			SEM ^a
	1	2	3	
OM, % DM				
Pasture 1	80	76	78	0.7
Pasture 2	81	84	82	0.7
Pasture 3	80	78	79	0.7
CP, % DM				
Pasture 1	8.6	5.6	6.8	0.61
Pasture 2	9.2	5.5	7.3	0.61
Pasture 3	9.3	6.1	7.9	0.61
NDF, % DM				
Pasture 1	62	63	60	1.3
Pasture 2	62	64	58	1.3
Pasture 3	61	62	59	1.3
ADF, % DM				
Pasture 1	39	40	37	0.9
Pasture 2	38	38	36	0.9
Pasture 3	38	37	37	0.9
C32, mg/kg DM ^b				
Pasture 1	23	35	33	---
Pasture 2	30	36	33	---
Pasture 3	27	33	35	---
C33, mg/kg DM ^c				
Pasture 1	66	53	60	---
Pasture 2	72	55	74	---
Pasture 3	55	54	66	---

^an = 3, except for Year 1 where n = 2; therefore, the largest SEM is presented.

^bC32 = dotriacontane (C₃₂H₆₆).

^cC33 = tritriacontane (C₃₃H₆₈).

Northern Great Basin Experimental Range. In yr 1, masticate CP reached 9%, which was considerably higher than expected. This is largely the result of late-season rain initiating cool-season grass regrowth (data not shown).

Cow Performance

Cow BW and BCS change were greater ($P \leq 0.03$) for supplemented treatments compared with CON (Table 2). No change in BW or BCS ($P \geq 0.14$) was noted because of supplementation frequency. A trend for year effects was observed for BW and BCS change ($P = 0.06$ and 0.08 , respectively). Body weight change was 35 ± 23 , 28 ± 38 , and 48 ± 26 kg for yr 1, 2, and 3, respectively; however, no differences were observed for the effect of pasture on BW and BCS change ($P = 0.11$ and 0.41 , respectively). The variability in yearly data for cow BW and BCS change indicates that livestock producers need to make annual decisions regarding the supplementation of grazing livestock in the northern Great Basin, as forage CP concentration may vary from year to year. Estimated ruminally degraded intake protein (DIP) balance was -197 , -113 , and -68 g/d for CON, D, and 6D, respectively, assuming a microbial efficiency of 11% (Model 1; NRC, 1996) and cool-season forage DIP values reported by Bohnert et al. (2002b). The DIP balance

mirrored the response observed for performance. Although calculated DIP balance was negative for all treatments, supplemental DIP provided through the CSM may have contributed to improved performance for supplemented treatments. The estimated metabolizable protein (MP) balance was 303, 266, and 216 g/d for CON, D, and 6D, respectively. However, when the negative DIP balances are subtracted from the estimated MP balances, the adjusted MP balances also mirror the observed performance (106, 153, and 148 g/d for CON, D, and 6D, respectively; MP balance assumes that DIP requirements are met).

Ruminants consuming low-quality forage and supplemented as infrequently as once per week consistently exhibit BW and BCS changes similar to animals receiving daily supplementation (McIlvain and Shoop, 1962; Huston et al., 1999b; Bohnert et al., 2002b). McIlvain and Shoop (1962) supplemented cows grazing dormant winter range 7 d/wk, every third day, or 1 d/wk with CSM and noted similar BW gains among treatments. Likewise, Huston et al. (1999b) supplemented CSM to beef cows consuming low-quality native range in western Texas 7, 3, or 1 d/wk. Protein supplementation decreased BW loss by 67 to 83%, with no differences due to supplementation frequency. In an experiment by Bohnert et al. (2002b), cows consuming 5% CP meadow hay were supplemented with DIP or undegradable intake protein daily, once every 3 d, or once every 6 d. They noted that supplementation frequency did not affect BW or BCS gain. In contrast to these results, other researchers (Beaty et al., 1994; Farmer et al., 2001) reported that infrequent supplementation increased cow BW and BCS loss compared with daily supplementation. Beaty et al. (1994) fed a mixture of soybean meal and sorghum grain daily or three times per week to cows consuming wheat straw (3% CP). They reported precalving BW loss was decreased by 16% for daily supplementation compared with infrequent supplementation but was not different for cow BW and BCS change at weaning. Similarly, Farmer et al. (2001) fed a 43% CP supplement 7, 5, 3, or 2 d/wk to cows grazing tallgrass prairie (4% CP) and reported that infrequent supplementation linearly increased cow BW loss from December 7 until calving. Nonetheless, BCS change was unaffected by supplementation frequency during the same period. It is important to note that in both the aforementioned trials, magnitude of cow BCS and/or BW difference attributed to supplementation frequency tended to be relatively small and was not consistent throughout the trials.

The ability of ruminants to maintain BW and BCS as protein supplementation frequency decreases is probably due to similar digested N retained compared with daily supplementation (Bohnert et al., 2002b). Possible mechanisms for this response include increased urea-N removal from the blood by the portal-drained viscera as supplementation frequency decreases, increased permeability of the gastrointestinal tract to urea-N as the N content of the diet decreases between

Table 2. Effect of protein supplementation frequency on performance, behavior, fecal output, dry matter intake, dry matter digestibility, harvest efficiency, supplement intake, and supplement intake variability of cows grazing native range in the northern Great Basin

Item	Treatment ^a			SEM ^b	P-value ^c	
	CON	D	6D		Con vs. Supp.	D vs. 6D
Initial BW, kg	470	465	468	---	---	---
BW change, kg	17	51	43	2	0.01	0.14
Initial BCS	4.67	4.63	4.67	---	---	---
BCS change	0.01	0.45	0.32	0.06	0.03	0.24
Grazing time, h/d	9.57	7.08	7.87	0.36	0.04	0.26
Distance traveled, m/d	5,917	5,823	5,903	160	0.81	0.76
Maximum distance from water, m/d	1,912	1,919	1,760	105	0.63	0.40
Distribution, % ^d	70	69	67	2	0.42	0.40
Fecal output, g DM·kg BW ⁻¹ ·d ⁻¹	10.7	10.9	9.8	0.5	0.62	0.27
DMI, g·kg BW ⁻¹ ·d ⁻¹	24.9	21.6	18.6	1.8	0.16	0.36
DM digestibility, %	50.7	49.4	45.3	3.7	0.53	0.51
Harvest efficiency, g DMI·kg BW ⁻¹ ·min grazing ⁻¹	0.045	0.053	0.042	0.006	0.75	0.32
Supplementation events frequented, %	---	66	70	12	---	0.82
Estimated supplement intake, g DM·kg BW ⁻¹ ·d ^{-1e}	---	0.9	1.1	0.2	---	0.58
CV for supplement intake, % ^f	---	28	28	7	---	0.99

^aCON = control; D = supplementation everyday; 6D = supplementation every sixth day (least squares means).

^bn = 3.

^cCon vs. Supp = control vs. supplemented treatments; D vs. 6D = daily supplementation vs. every sixth day supplementation.

^dDistribution = percentage of ha occupied·pasture⁻¹·yr⁻¹.

^eEstimated using an algebraic approach (Galyean, 1993).

^fSupplement offered to provide 0.91 and 5.46 kg (DM)·cow⁻¹·supplementation event⁻¹ for D and 6D treatments, respectively.

supplementation events, and changes in renal regulation that decrease urinary N excretion as dietary N decreases between supplementation events (Krehbiel et al., 1998; Marini and Van Amburgh, 2003). Changes in renal regulation of urea-N excretion in ruminants fed low-quality forage and supplemented infrequently may assist in maintaining N status and ruminal fermentation through increased recycling of N to the gastrointestinal tract (Krehbiel et al., 1998; Bohnert et al., 2002b).

Cow Behavior and Distribution

No treatment × day interactions occurred ($P = 0.61$) for grazing time; therefore, only treatment means are presented. Grazing time was 2.1 h greater ($P = 0.04$) for CON compared with supplemented treatments, with no difference ($P = 0.26$) due to supplementation frequency (Table 2). Variable results have been reported regarding the effect of supplementation on grazing time. Cows grazing dormant tallgrass prairie and supplemented with alfalfa decreased grazing time by 10% compared with unsupplemented individuals (Yelich et al., 1988). Similarly, Barton et al. (1992) demonstrated that providing CSM to steers grazing intermediate wheatgrass decreased time spent grazing by 1.5 h/d. Krysl and Hess (1993) reported in a review that supplementation seemed to decrease grazing time of cattle by 1.5 h/d. In contrast, Bodine and Purvis (2003) noted that steers grazing dormant, native tallgrass prairie and offered soybean meal 5 d/wk exhibited no difference in grazing time compared with unsupplemented con-

trols (8.6 and 9.1 h/d, respectively). Similarly, Brandyberry et al. (1992) reported that cows grazing dormant native range (6% CP) and supplemented daily or on alternative days with alfalfa hay or alfalfa pellets did not modify their grazing behavior. Results from our trial indicate that protein supplementation decreased grazing time by approximately 2 h/d, which agrees with the majority of literature. Differences between our trial and Bodine and Purvis (2003) may be due to pasture size (810 vs. 130 ha), which may have affected herd dynamics and/or social facilitation.

Distance traveled and maximum distance from water did not exhibit treatment × day interactions ($P \geq 0.06$), so overall treatment means are presented. Distance traveled ($5,881 \pm 160$ m/d) and maximum distance from water ($1,864 \pm 105$ m/d) were not affected ($P \geq 0.40$) by protein supplementation or supplementation frequency (Table 2). Additionally, cow distribution ($69 \pm 2\%$ ha occupied·pasture⁻¹·yr⁻¹) was not affected by protein supplementation or supplementation frequency ($P \geq 0.40$; Table 2).

Although strategic placement of a protein supplement can affect livestock distribution (Bailey et al., 2001), little research has evaluated the effects of protein supplementation and supplementation frequency on livestock distribution. Adams (1985) reported that steers grazing Russian wild ryegrass (7% CP) and supplemented with corn traveled 500 m/d further than unsupplemented steers. In contrast, Barton et al. (1992) noted that steers supplemented with CSM did not spend more time walking than unsupplemented steers grazing intermediate wheatgrass pasture. Martin and

Ward (1973) reported similar results, noting that on a semi-desert grass-shrub range, meal salt supplementation resulted in no affect on pasture utilization and cow distribution. Differences among trials for livestock distribution may be attributed to differences in grazing behavior because of supplement type (DelCurto et al., 1990) as well as differences among trial locations in forage quantity and quality (native vs. introduced pastures). In research conducted at the Northern Great Basin Experimental Range, Brandyberry et al. (1992) reported that cows grazing native range (6% CP) and supplemented with alfalfa hay or alfalfa pellets daily or every other day traveled the same distance (9920 m/d). Melton and Riggs (1964), however, observed that cows supplemented infrequently with cottonseed cake grazed more widely over winter range than cows supplemented daily. Our results agree with those reported by Brandyberry et al. (1992), suggesting that supplementation frequency does not affect distance traveled, maximum distance from water, or cow distribution in the northern Great Basin. Differences between our results and those of Melton and Riggs (1964) could be attributed to disparities in location (southwestern United States vs. northern Great Basin), supplement placement within pasture, pasture topography, and/or herd age. In addition, behavioral data reported by Melton and Riggs (1964) were anecdotal in nature, whereas our trial was designed to quantify distribution affects.

Dry Matter Intake and Digestibility

Fecal output (10.5 ± 0.5 g DM·kg BW⁻¹·d⁻¹) and DMI (21.7 ± 1.8 g·kg BW⁻¹·d⁻¹) were unaffected ($P \geq 0.16$) by protein supplementation or supplementation frequency (Table 2); however, DMI tended to decrease for supplemented treatments compared with CON ($P = 0.16$), which is consistent with responses reported for grazing time. The inability to detect a treatment effect may be because of the innate variability associated with the use of alkanes to determine DMI combined with the limited replications due to logistical constraints. Berry et al. (2000) described the variability associated with predicting DMI from alkane controlled-release capsules and fecal grab sampling. They concluded that intake estimates were accurate; however, they reported that the use of alkanes as a dual marker obviously results in greater variability in intake and digestibility data compared with the use of individual markers. Other efforts have suggested that protein supplementation increases DMI of low-quality forage (McCollum and Galyean, 1985; Köster et al., 1996; Bandyk et al., 2001). One possible explanation for our lack of a treatment effect could be related to NDF intake. Mertens (1985, 1994) proposed that NDF intake might be the most important factor influencing forage intake of ruminants fed low-quality forage, suggesting that a forage intake response to protein supplementation may be expected when NDF intake is less than 12.5 g·kg BW⁻¹·d⁻¹. In our trial, calculated NDF intake of control cows was

approximately 15 ± 1.1 g·kg BW⁻¹·d⁻¹ based on an average DMI of 24.9 g·kg BW⁻¹·d⁻¹ (Table 2) and an average masticate NDF of 61% (Table 1). Therefore, we did not expect to alter DMI with supplementation.

Several researchers have reported no affect of supplementation frequency on DMI (Coleman and Wyatt, 1982; Krehbiel et al., 1998; Huston et al., 1999a). Coleman and Wyatt (1982) noted that steers fed range hay (8% CP) and supplemented with CSM every other day or once every 4 d had similar DMI to steers supplemented daily. Additionally, Krehbiel et al. (1998; 7.5% CP brome grass hay) and Huston et al. (1999a) reported no difference in forage intake by ewes supplemented infrequently compared with daily supplementation. In contrast, other researchers have demonstrated a decrease in forage intake as supplementation frequency decreased (Beaty et al., 1994; Huston et al., 1999b; Bohnert et al., 2002b). Steers consuming wheat straw (3% CP) and supplemented three times per week consumed 17% less straw than individuals supplemented daily (Beaty et al., 1994). Similarly, Huston et al. (1999b) noted a decrease in forage intake of 38 and 27% with supplementation 3 d/wk and once per week, respectively, compared with daily supplementation. Bohnert et al. (2002b) reported a linear decrease in hay intake (13%) as supplementation frequency decreased (supplementation daily vs. once every 3 d vs. once every 6 d) for lambs consuming low-quality meadow hay (5% CP). Huston et al. (1999b) and Bohnert et al. (2002b) suggested that decreased forage intake might be due to substitution of supplement DM for forage DM by infrequently supplemented individuals, with the effect most pronounced on the day of a supplementation event.

Dry matter digestibility ($48 \pm 4\%$) was not affected ($P \geq 0.51$) by protein supplementation or supplementation frequency (Table 2). Although some researchers have noted an increase in DM digestibility of low-quality forage when ruminants are supplemented with protein (McCollum and Galyean, 1985; Caton et al., 1988; Bohnert et al., 2002a), the majority of these responses occurred when forage CP was below 6% of DM. Peterson (1987) suggested that increases in the digestibility of low-quality forage due to protein supplementation may be largely the result of improved N availability for the ruminal microflora. Research conducted by Mathis et al. (2000) demonstrated that when steers were infused ruminally with sodium caseinate, total-tract digestion of OM and NDF was not affected when forage CP was 6 to 8%; however, when forage CP decreased to 4%, total-tract digestion of OM and NDF increased with sodium caseinate infusion. In our trial, masticate CP averaged 7.4%, which may have been sufficient to maintain ruminal fermentation and digestion, even though calculated DIP balances were negative. The lack of a protein supplementation effect on DM digestibility also could explain our lack of an affect on DMI, but the supplemental CSM seems to have provided additional

protein (both DIP and undegradable intake protein), which resulted in improved livestock performance.

Supplementation frequency has had no effect on DM digestibility in several studies (Coleman and Wyatt, 1982; Hunt et al., 1989; Bohnert et al., 2002a). Similarly, Hunt et al. (1989) found no difference in NDF and ADF in situ disappearance in steers supplemented infrequently with CSM, while consuming low-quality fescue hay (6.6% CP). Bohnert et al. (2002a) evaluated ruminal, postruminal, and total-tract OM disappearance as affected by supplementation frequency. Decreasing supplementation frequency had no effect on OM digestibility in steers fed low-quality meadow hay (5% CP) and supplemented once, three times, or six times per week; however, variable results in total-tract disappearance of nutrients have been observed (Beaty et al., 1994; Farmer et al., 2001). Beaty et al. (1994) reported that reducing supplementation frequency increased DM and NDF digestion by steers consuming wheat straw (3% CP) and supplemented once or three times per week. In contrast, Farmer et al. (2001) noted that steers consuming tallgrass prairie (5% CP) and supplemented as infrequently as once per week had decreased total tract OM disappearance as supplementation frequency decreased. They proposed that this effect may have been due to altered ruminal fermentation by infrequent supplementation, which was supported by a quadratic decrease in ruminal fluid dilution rate as supplementation frequency decreased. The magnitude of change in OM disappearance was small (5%), however, so the biological relevance of the decrease in OM disappearance may be questionable. Our results are similar to those of Bohnert et al. (2002b), who noted no difference in total tract DM, OM, or NDF digestibility in lambs consuming low-quality forage and supplemented with protein infrequently.

Harvest efficiency was unaffected ($P \geq 0.32$) by protein supplementation or supplementation frequency (Table 2). Although no other research has evaluated the affects of supplementation frequency on harvest efficiency, Krysl and Hess (1993) reported in a review that protein supplementation increases harvest efficiency from 8 to 60% compared with no supplementation. When we noted a decrease in grazing time and no effect on DMI, we were surprised to find no effect of protein supplementation on harvest efficiency. This may be due to the numerical decrease in DMI as supplementation frequency decreased in conjunction with the decrease in grazing time. Additionally, DMI and grazing time estimates were not evaluated simultaneously (to avoid collecting GPS data at the same time of conducting fecal collections), which may have altered our ability to detect differences in harvest efficiency.

Variability in Supplement Intake

The percentage of supplementation events frequented ($68 \pm 12\%$) was not affected by supplementation frequency ($P = 0.82$; Table 2), suggesting that group

feeding behavior was not influenced by decreasing the frequency of supplementation events from D to 6D. This response is similar to the response reported for grazing time. Even though all cows may not have been present for all supplementation events, the cows that were present always consumed the entire quantity of supplement within 20 min. Control cows did not respond to the audio cue, most likely because of the distance between watering/supplementation locations across pastures (minimum of 3 km). In contrast, McIlvain and Shoop (1962) and Melton and Riggs (1964) reported anecdotal observations that more frequently supplemented cows seemed to anticipate supplementation events more consistently than those supplemented less frequently. Similarly, Beaty et al. (1994) reported that as supplementation frequency decreased, cow proximity to the feeding area before a supplementation event decreased. Differences among trials may be attributed to our ability to monitor individual animals compared with case studies or observational data from an entire herd. Additionally, we provided an audio cue to entice cows to a supplementation event, which was not provided in the previously mentioned studies.

Calculated supplement intake was 99 and 121% of supplement offered $\cdot \text{cow}^{-1} \cdot \text{d}^{-1}$ for D and 6D, respectively ($P = 0.58$; Table 2). It has been reported that age plays a role in feeding behavior and dominance within a mixed-age cowherd (Sowell et al., 1999). We were unable to test the influence of age on supplement intake and intake variability because of experimental design; however, cows were stratified by age when allotted to treatments to minimize potential treatment effects due to cow age.

The CV for supplement intake was not different ($P = 0.99$) between D and 6D treatments (Table 2). This contrasts with results reported by Huston et al. (1999b), who noted that cows supplemented infrequently (three times weekly or once weekly) exhibited approximately 33% less variation in supplement intake than cows supplemented daily. This reported decrease in the CV for supplement intake was attributed to the increased quantity of supplement offered with less frequent supplementation. Foot and Russel (1973) report that the CV for supplement intake decreased from 36 to 13% when supplement offered to ewes was increased from 100 to 453 g/d. Additionally, Kahn (1994) found that the CV for supplement intake by sheep decreased by 10% when supplement offered was increased from 55 to 110 g/d. Infrequent supplementation increases the amount of supplement offered per supplement event, potentially allowing individual animals an increased opportunity to consume supplement. It is not clear why the results in the current study differed from the aforementioned studies. One possible explanation is that the supplement intake and variability in supplement intake data were measured at only one point in time and included a small subset of study animals (10% of herd). However, we are unaware of alternative tech-

niques available for the monitoring of variability in supplement intake at the scale we used.

Implications

Infrequent supplementation of crude protein to cows grazing low-quality forage resulted in animal performance and grazing behavior similar to that of cows receiving supplement daily. Infrequent protein supplementation is a management alternative that can lower labor and fuel costs associated with supplementation of cows grazing native range in the northern Great Basin. Annual decisions regarding supplementation of grazing livestock may be necessary, as climate and precipitation patterns may affect forage nutritive value, resulting in variability in livestock performance between growing seasons.

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